

Some Dietary Fibers Reduce the Absorption of Carotenoids in Women¹

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ABSTRACT Dietary fiber may be partly responsible for the lower bioavailability of carotenoids from food than from purified supplements. Due to the lack of detailed information available, we investigated the effects of different kinds of dietary fiber on the absorption of carotenoids and α -tocopherol. Six healthy young women received an antioxidant mixture consisting of β -carotene, lycopene, lutein, canthaxanthin and α -tocopherol together with a standard meal. The meal did not contain additional dietary fiber or was enriched with pectin, guar, alginate, cellulose or wheat bran ($0.15 \text{ g} \cdot \text{kg body weight}^{-1}$). The increases in plasma carotenoid and α -tocopherol concentrations were followed over 24 h, and the areas-under-curves ($\text{AUC}_{24\text{h}}$) were calculated. The mean $\text{AUC}_{24\text{h}}$ of β -carotene was significantly ($P < 0.05$) reduced by the water-soluble fibers pectin, guar and alginate with a mean decrease of 33–43%. All tested fibers significantly reduced the $\text{AUC}_{24\text{h}}$ of lycopene and lutein by 40–74% ($P < 0.05$). The dietary fiber effect on the $\text{AUC}_{24\text{h}}$ of canthaxanthin was almost significant ($P = 0.059$) and there was no effect on the $\text{AUC}_{24\text{h}}$ of α -tocopherol. We conclude that the bioavailability of β -carotene, lycopene and lutein given within a mixed supplement is markedly reduced by different kinds of dietary fiber.

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Because of their antioxidant activity, α -tocopherol and carotenoids have attracted much scientific attention. α -Tocopherol has been shown in vitro and in vivo to inhibit oxidative damage especially in LDL and thus may lower or even prevent atherosclerotic processes induced by oxidized LDL (Diaz et al. 1997, Esterbauer et al. 1992, Holvoet and Collen 1998). According to epidemiologic studies, a diet rich in carotenoid-containing foods is associated with a number of health benefits, i.e., reduced risk of cancer, cardiovascular disease, macular degeneration and cataract formation (Biesalski et al. 1997, Rock 1997). The antioxidant activity of carotenoids shown in vitro was studied in randomized intervention trials by administration of pure β -carotene. However, these studies gave no convincing or even negative results regarding the risk of certain cancers and cardiovascular disease (Albanes et al. 1995, Biesalski et al. 1997, Rock 1997). Other carotenoids have not been tested. Possibly, β -carotene intake has to be considered as an indicator substance for a "healthy diet" with a high intake of fruit and vegetables. However, the modes of actions of carotenoids and α -tocopherol are diverse and have not been entirely clarified. They are also present intracellularly and may be involved in the regulation of gene expression or affect cell functions like inhibition of monocyte adhesion and platelet activation (Devaraj et al. 1996, Parker 1997, Rock 1997). Although specific activities are known, whether they are the explanatory mechanisms of action is unknown, and the role of interactions is still under discussion (Biesalski et al. 1997, Parker 1997).

Plasma β -carotene response to a test dose of β -carotene was much greater from a purified supplement than from a natural food source, such as carrots (Rao and Rao 1970). Also, β -carotene supplementation was demonstrated to impair the bioavailability of other carotenoids, possibly followed by biological effects (Kostic et al. 1995, Paetau et al. 1997).

Besides fat intake and the efficiency of extraction from the food matrix, the amount and type of dietary fiber in the diet seem to determine carotenoid bioavailability (Parker 1997, Rock 1997, Williams et al. 1998). However, detailed information on the effect of different kinds of dietary fiber on carotenoid absorption in humans is lacking. Only one study showed a distinct adverse effect of pectin on β -carotene absorption (Rock and Swendseid 1992). Dietary vitamin E may also affect carotenoid bioavailability (Mobarhan et al. 1994, Rock 1997, Xu et al. 1992). As stated for carotenoids, knowledge about dietary fiber effects on α -tocopherol bioavailability is scarce (Kayden and Traber 1993).

Therefore, in the present investigation the effects of various fibers on plasma response to supplemented carotenoids and α -tocopherol as an indicator of absorption were tested. The substances were administered as a mixture of the fat-soluble antioxidants together with a test meal enriched with dietary fiber.

METHODS

Subjects. The investigation was conducted in six young, non-pregnant women (ages 26–29 y). They were free of acute or chronic disease and took no nutrient supplements before the study. All of them showed normal body weight with a body mass index of 19.3–21.7 kg/m^2 (Table 1), were nonsmokers and did not take oral contraceptives. All participants gave informed consent prior to the

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TABLE 1

Characteristics of the six women participating in a study to determine the effect of different kinds of dietary fiber on the bioavailability of supplemented carotenoids and α -tocopherol^{1,2}

	Subject no.					
	I	II	III	IV	V	VI
Age, y	26	27	27	26	29	26
Body height, m	1.67	1.69	1.79	1.68	1.61	1.67
Body weight, kg	53.9 \pm 0.3	60.6 \pm 0.2	69.0 \pm 0.1	57.3 \pm 0.3	52.6 \pm 0.1	60.6 \pm 0.2
Body mass index, kg/m ²	19.3 \pm 0.1	21.2 \pm 0.1	21.5 \pm 0.02	20.3 \pm 0.1	20.3 \pm 0.04	21.7 \pm 0.1
Body fat mass ³ , kg	11.8 \pm 0.1	15.7 \pm 0.2	17.7 \pm 0.1	11.3 \pm 0.3	9.4 \pm 0.2	15.7 \pm 0.2
Fasting (0 h) plasma concentrations:						
Total cholesterol, mmol/L	3.79 \pm 0.10	4.26 \pm 0.17	3.62 \pm 0.16	5.34 \pm 0.08	4.49 \pm 0.11	4.23 \pm 0.18
Triacylglycerol, mmol/L	0.56 \pm 0.05	0.58 \pm 0.04	0.50 \pm 0.03	0.82 \pm 0.04	0.75 \pm 0.05	0.76 \pm 0.04

¹ Values are means \pm SEM over all six test days.

² The combined supplement was consumed with a standard meal with or without added dietary fiber.

³ By bioelectrical impedance analysis, using a TVI-10 Body Composition Analyzer (Danninger Medical Technology, Inc., Columbus, OH).

beginning of the study. The procedures followed were in accord with the Helsinki Declaration of 1975 as revised in 1983.

Experimental design. Three days before the study (prephase), the subjects consumed a diet low in tocopherol and carotenoids to exclude plasma enrichment with antioxidants originating from food consumed before the test day. On the test day, the six subjects were offered a standard meal and an antioxidant supplement. The standard meal contained either no additional dietary fiber or one of the five types of dietary fiber to be tested. Each subject (unaware of the type of dietary fiber) received a different kind of dietary fiber within a test day. Every test day was followed by a wash-out period of at least 2 wk. All subjects completed the study, thus each received each type of fiber.

Dietary regimen, dietary fiber and antioxidant supplement. During the 3-d prephase, the subjects were advised to consume a diet low in carotenoids and tocopherol according to detailed instructions of a dietitian and a list of foods to be avoided. Calculations of dietary intake were based on dietary protocols (estimated record) completed during the study prephases using a German standard food composition data base (DFL 1986) after addition of carotenoid data from three more sources [most data from Mangels et al. 1993, and some additional data from Heinonen et al. (1989) and Ollilainen et al. (1988)]. The subjects' compliance was also monitored by means of plasma analysis of α -tocopherol and carotenoids before and after the prephase periods.

On the test day, the fasting (for at least 10 h) subjects were offered a standard meal consisting of chocolate rice pudding for breakfast. In preliminary studies, chocolate rice pudding was found to be suitable to disguise the use of fiber to the subjects. The standard meal (including the antioxidant supplement) contained 27% of its energy as fat, 59% as carbohydrates and 14% as protein. The meal provided 20% of the estimated daily energy requirement of each subject (REE \cdot 1.5), with the other 80% of energy given in six portions every 2 h in the form of a fiber-free liquid diet (Nutricomp F; B. Braun Melsungen AG, Melsungen/Germany: 24% of energy as fat, 59% as carbohydrates, 17% as protein; 7.5 mg α -tocopherol/L; no carotenoids).

The standard meal was given without added dietary fiber or enriched with one of the five different types of dietary fiber. Either pectin (GENU pectin, type X5114, citrus pectin with a degree of esterification of 70%; Copenhagen Pectin A/S, Lille Skensved/DK), guar (MEYPRO GUAR, type CSA 200/50, Welding GmbH & Co, Hamburg/Germany), alginate (Manucol DMF, Kelco International, Waterfield/UK), cellulose (Vitacel[®], type L 600, J. Rettenmaier & Söhne, Ellwangen-Holzmühle/Germany), or wheat bran (Dr. Kousa Weizenkleie, Milupa AG, Friedrichsdorf/Germany) were added to the standard meal, amounting to 0.15 g \cdot kg body weight (bw)⁻¹. Table water was used to obtain comparable consistencies of the meals.

The subjects were advised to consume an antioxidant supplement containing all-trans- β -carotene (0.4 mg \cdot kg bw⁻¹; Hoffmann-La Roche, Grenzach-Wyhlen/Germany), lycopene (0.7 mg \cdot kg bw⁻¹;

LycorRed Natural Products Industries, Beer-Sheva/Israel), canthaxanthin (0.2 mg \cdot kg bw⁻¹, Hoffmann-La Roche), lutein (0.4 mg \cdot kg bw⁻¹; Kemin Industries, Des Moines, IA), and α -tocopherol (1.4 mg \cdot kg bw⁻¹; Hoffmann-La Roche). An ultrasonic bath (5 min 40°C) was used to get β -carotene, canthaxanthin and α -tocopherol homogeneously distributed in 3 mL of cream, while the other two carotenoids were distributed in olive oil (2 g). The combined mixtures were filled into a small consumable cup to ensure complete intake without any waste. The subjects ate the standard meal and the antioxidant supplement within 10 min.

Plasma preparation and analysis. Blood samples were drawn into tubes containing the antioxidants EDTA (1.6 g/L blood) and TROLOX (1 μ mol/L blood; both from Sigma, Deisenhofen/Germany) before as well as 2, 4, 6, 7, 8, 9, 10 and 24 h after the test meal. The plasma samples were stored at -24°C for a maximum of 4 wk until analysis.

Plasma carotenoid and α -tocopherol concentrations were analyzed by HPLC according to a described method (Hess et al. 1991). Briefly, after protein precipitation hexane was used for extraction. The hexane layer was evaporated under vacuum and redissolved in 200 μ L of HPLC eluent. Twenty μ L were injected to a reversed-phase C-18 column (precolumn ultra-sphere ODS, 4.5 mm \times 45 mm column ultrasphere ODS, 4.5 mm \times 150 mm; Beckmann München, Germany). Analysis of α -tocopherol and carotenoids was performed by detecting absorbance at 292 and 450 nm, respectively, after elution with acetonitrile/dichloromethane/methanol (7:2:1, vol/vol, vol; 1.2 mL/min, 18°C). Absorbance was analyzed with an UV/VIS detector UVD 340 S from Gynkoteck (Germering/Germany). Calculations were made using the internal standard method and relative response factors. β -Apo-8'-carotenoic acid ethyl ester (carotenoid quantification) and DL- α -tocopheryl acetate (α -tocopherol quantification) were added as internal standards. Recovery of the substances after enrichment of serum samples was within 94 and 107% with a coefficient of variation < 2.3% (n = 5).

Plasma total cholesterol and triacylglycerol concentrations were determined enzymatically using test kits (Triglycerides GPO-PAP Cholesterol CHOD-PAP, both Boehringer Mannheim GmbH Mannheim/Germany).

Areas-under-curves (AUC)³ calculation and statistics. As a measure of absorption efficiency, the areas under the plasma enrichment curves of the antioxidants were integrated by trapezoidal approximation over a time period of 24 h (AUC_{24h}).

The results are given as means and (SEM). Statistical analysis was performed with SPSS, Vers. 7.5 (SPSS, Chicago, IL). For analysis of variance of the AUC_{24h}-values, a two-factorial model with the factors "person" and "dietary fiber" was applied. Comparisons of means were made with the Student-Newman-Keuls-test (SNK-test) at an

³ Abbreviation used: AUC_{24h}, areas-under-curves calculated over 24 h¹.

TABLE 2

Dietary intake of β -carotene, lycopene, lutein, canthaxanthin and α -tocopherol during a 3-d period in carotenoids and α -tocopherol as well as their fasting plasma concentrations before and after the diet in healthy young women¹

	Dietary intake	Plasma concentrations		
		Before diet	After diet ²	Change
	$\mu\text{g/d}$	$\mu\text{mol/L}$		%
β -Carotene	277 \pm 10	1.34 \pm 0.11	1.20 \pm 0.07*	-10.5
Lycopene	0	0.56 \pm 0.05	0.44 \pm 0.03*	-21.5
Lutein	136 \pm 11	0.28 \pm 0.03	0.21 \pm 0.01*	-25.5
Canthaxanthin	0	0	0	0
α -Tocopherol	4700 \pm 190	20.54 \pm 0.88	19.58 \pm 0.57*	-6.7

¹ Values are means \pm SEM, $n = 6$.

² After-diet value is equal to 0 h-value on test day.

* Significantly different means than before diet, $P \leq 0.05$ (paired t -test).

α -level of 5%. The paired t -test was used for comparison of fasting plasma concentrations of antioxidants before vs. after a diet low in carotenoids and α -tocopherol with a significance level of $P < 0.05$.

RESULTS

Examination of the 3-d low-tocopherol and low-carotenoid diet revealed mean intake data for α -tocopherol and the sum of carotenoids of 4.7 ± 0.2 mg/d and 427 ± 16 $\mu\text{g/d}$, respectively (Table 2). On average, energy intake amounted to 8.23 ± 0.15 MJ/d (1967 ± 35 kcal/d); fat contributed 31%, carbohydrates 56%, and protein 14% of total energy intake. The diet provoked a significant reduction of the carotenoid and α -tocopherol plasma concentrations (Table 2), indicating good diet compliance of all subjects.

Fasting plasma total cholesterol and triacylglycerol concentrations were 4.30 ± 0.11 and 0.68 ± 0.02 mmol/L, respectively, and showed no significant differences among test days. Fasting plasma concentrations of each subject over all test days are given in Table 1. Within test days, mean plasma cholesterol concentrations ranged between 4.06 and 4.26 mmol/L estimated at nine time points over a period of 24 h; mean plasma triacylglycerol concentrations ranged between 0.64 and 0.80 mmol/L. None of the subjects responded markedly

differently in plasma cholesterol and triacylglycerol. Analysis of variance (multifactorial) revealed no significant effect of plasma total cholesterol and triacylglycerol concentrations on plasma carotenoid and α -tocopherol levels. Accordingly, the plasma concentrations of carotenoids and α -tocopherol presented are not corrected for the plasma lipid concentrations.

After intake of the combined antioxidant supplement and the standard meal without added dietary fiber, the plasma concentrations of the supplement components increased and reached maximum values 7–8 h, later, however, the plasma β -carotene increase continued throughout the observation period of 24 h. Consistent with the results of preliminary studies, plasma concentrations of β -carotene showed their maximum approximately 24 h after intake of the antioxidant supplement ($n = 2$), and later measurements (30 h) indicated no additional increase. Addition of any kind of dietary fiber to the standard meal resulted in decreased mean plasma responses of carotenoids and α -tocopherol over 24 h. The AUC were calculated to give a measure of the relative effect of the different kinds of dietary fiber (Table 3). Meal enrichment with the water-soluble fibers pectin, guar, and alginate significantly decreased the AUC_{24h} for β -carotene by 42, 43 and 33%, respectively (Fig. 1). All kinds of dietary fiber significantly reduced the plasma response curves of lycopene and lutein (Table 3). Expressed as the percentage of the AUC_{24h} of the test conducted without added dietary fiber, addition of dietary fiber decreased the relative absorption of lycopene and lutein by 40–74%. The dietary fiber effect on the AUC_{24h} of canthaxanthin failed to reach statistical significance ($F = 0.059$, two-factorial ANOVA, Table 3). The plasma response curves of α -tocopherol were not affected by addition of dietary fiber.

The AUC results for β -carotene, canthaxanthin and α -tocopherol were significantly affected by the study subjects (Table 4). Given the lack of a dietary fiber effect on the AUC results for canthaxanthin and α -tocopherol, β -carotene was the most interesting compound affected by both factors "subject" and "dietary fiber". The β -carotene response of subject III especially was much different from the others (Fig. 2). When the results of subject III were excluded from the statistical analysis, the effects of cellulose and wheat bran on the β -carotene response were significant.

DISCUSSION

The absorption of carotenoids and tocopherol takes place in the intestinal mucosa, and the uptake appears to be by

TABLE 3

Dietary fiber effect on the areas under the curves calculated from the change in plasma concentrations of β -carotene, lycopene, lutein, canthaxanthin and α -tocopherol during the 24 h after their combined supplementation in healthy young women^{1,2}

	Dietary fiber group					
	Without dietary fiber	Pectin	Guar	Alginate	Cellulose	Wheat bran
	AUC _{24h} , $\mu\text{mol} \cdot \text{h} \cdot \text{L}^{-1}$					
β -Carotene	8.67 \pm 1.03 ^a	5.00 \pm 1.32 ^b	4.97 \pm 0.77 ^b	5.83 \pm 1.61 ^b	6.91 \pm 1.32 ^{a,b}	7.08 \pm 1.36 ^{a,b}
Lycopene	1.89 \pm 0.34 ^a	1.12 \pm 0.21 ^b	0.65 \pm 0.06 ^b	0.71 \pm 0.20 ^b	0.68 \pm 0.16 ^b	0.94 \pm 0.15 ^b
Lutein	0.44 \pm 0.07 ^a	0.27 \pm 0.08 ^b	0.22 \pm 0.03 ^b	0.19 \pm 0.04 ^b	0.20 \pm 0.05 ^b	0.12 \pm 0.03 ^b
Canthaxanthin	4.49 \pm 0.28	3.75 \pm 0.44	3.88 \pm 0.28	3.70 \pm 0.28	4.16 \pm 0.43	4.50 \pm 0.21
α -Tocopherol	54.48 \pm 8.05	53.34 \pm 7.59	51.07 \pm 9.25	40.49 \pm 5.18	52.41 \pm 7.91	42.33 \pm 6.85

¹ Values are means \pm SEM, $n = 6$. ^{a,b} Means with no common letters differ significantly $P \leq 0.05$, SNK-test.

² The supplement was consumed with a standard meal with or without added dietary fiber.

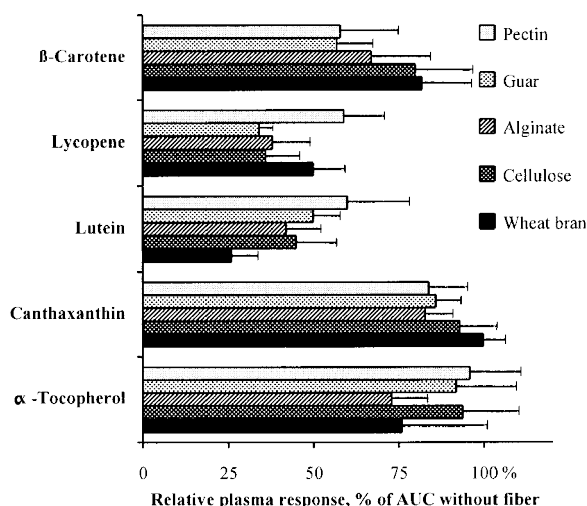


FIGURE 1 Effect of different kinds of dietary fiber on the relative plasma response (AUC_{24h} in % of AUC_{24h} without dietary fiber) of β -carotene, lycopene, lutein, canthaxanthin and α -tocopherol to their supplementation in healthy young women. Values are expressed as means \pm SEM, $n = 6$. The combined supplement was consumed with a standard meal with or without added dietary fiber. For significant differences between dietary fiber groups, see Table 3. AUC_{24h} , areas under curves calculated after following for 24 h.

passive diffusion along a concentration gradient between the mixed micelle and the mucosal cell. The formation of micelles is a necessary precondition implicating the importance of dietary fat intake for the absorption of carotenoids and vitamin E (Furr and Clark 1997, Kayden and Traber 1993, Williams et al. 1998). In the present investigation, fat intake on test days was kept constant with fat contributing 27% of the energy content of the test meal. During the 3-d study prephase, mean fat intake was 31% of energy intake with a very low variation. Besides this rather low contribution of fat, the high plasma concentrations of β -carotene (Table 2) also indicate, that the six young women usually consumed a rather "healthy diet" and apparently followed a "healthy life-style" (normal and constant BMI, nonsmokers, sports activities, no oral contraceptives). The increase in plasma levels after high β -carotene intake may continue up to several days (Furr and Clark 1997). In accordance with Van Vliet et al. (1995), a wash-out period of consuming low-carotenoid and low-tocopherol diet was found to be sufficient to ensure that the increase in plasma

carotenoid and α -tocopherol concentrations on the test day are not a consequence of a high intake on the days before (Table 2).

The literature describes a high intersubject variation for the plasma response to carotenoid (β -carotene) supplementation but a much lower intrasubject variation (Micozzi et al. 1992, O'Neill and Thurnham 1998). With the study design used, the problem of intersubject variation was markedly reduced and the SEM of the fasting plasma concentrations before test meal intake (Table 2) confirm a relatively low intrasubject variation between test days for all carotenoids and for α -tocopherol. However, a significant effect of the subjects on the AUC values (with and without dietary fiber addition) of β -carotene, canthaxanthin and α -tocopherol was found (Table 4). Most strikingly, subject III affected the mean plasma response for β -carotene, resulting in a nonsignificant effect of supplemental cellulose and wheat bran (Fig. 2).

The stage of menstrual cycle can affect plasma carotenoid and α -tocopherol concentrations in premenopausal women (Forman et al. 1998, Lanza et al. 1998). However, no data clearly indicate that the plasma response to a carotenoid supplement depends on the menstrual cycle phase or the fasting plasma concentrations affected by the menstrual cycle phase. Due to the lack of exact information on the menstrual cycle stage of the women in the present study, the evaluation of this factor as a confounding variable on plasma response values could not be performed.

Plasma response curves are widely used as an index of carotenoid bioavailability (Parker 1997). However, their use is limited by several factors like the problem of first-pass-metabolism of provitamin A carotenoids in the intestine or the recirculation of carotenoids between tissues and plasma. Moreover, to reach a distinct increase in plasma concentrations over baseline levels and to enable the calculation of the AUC, carotenoid doses far beyond the typical dietary intake are necessary and could have affected the results. As found by others (Furr and Clark 1997), β -carotene supplementation provoked a long-lasting increase in plasma concentrations, giving a different plasma enrichment curve as those found for the other antioxidants within 24 h. The AUC_{24h} results for β -carotene can be criticized for having included the plasma increase phase only and not having clearly identified the plasma peak nor included the decrease phase. However, Rock and Swendseid (1992) who followed the plasma concentration of β -carotene over 192 h found very similar results for the effect of pectin on the bioavailability of β -carotene as reported

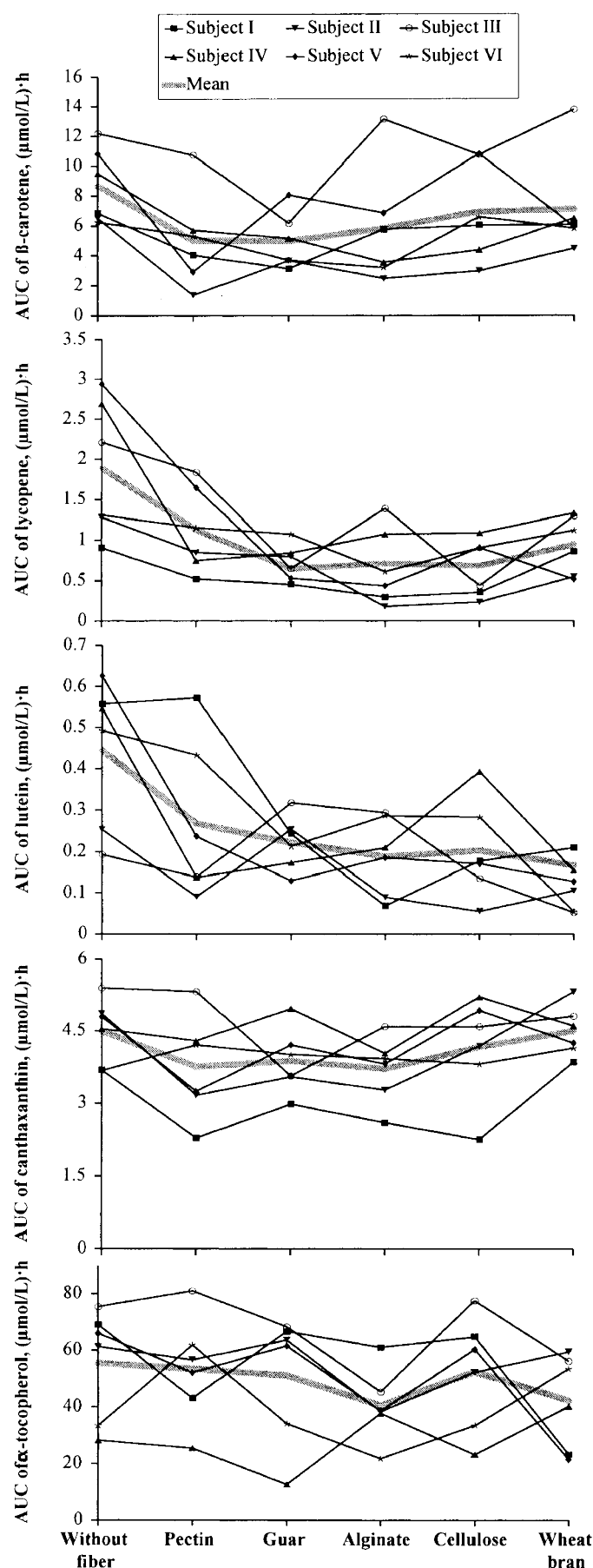
TABLE 4

Area under the curves for individual healthy young women calculated from the change in plasma concentrations of β -carotene, lycopene, lutein, canthaxanthin and α -tocopherol during the 24 h after their combined supplementation^{1,2}

	Subject					
	I	II	III	IV	V	VI
	AUC_{24h} , $\mu\text{mol} \cdot \text{h} \cdot \text{L}^{-1}$					
β -Carotene	$5.30 \pm 0.58^{b,c}$	3.57 ± 0.73^c	11.11 ± 1.11^a	$5.79 \pm 0.84^{b,c}$	7.55 ± 1.24^b	$5.13 \pm 0.56^{b,c}$
Lycopene	0.56 ± 0.11	0.65 ± 0.17	1.30 ± 0.28	1.29 ± 0.29	1.16 ± 0.40	1.03 ± 0.10
Lutein	0.30 ± 0.09	0.14 ± 0.04	0.18 ± 0.04	0.27 ± 0.07	0.25 ± 0.25	0.29 ± 0.06
Canthaxanthin	2.94 ± 0.28^b	4.06 ± 0.36^a	4.71 ± 0.27^a	4.60 ± 0.18^a	4.20 ± 0.25^a	3.96 ± 0.08^a
α -Tocopherol	$54.58 \pm 7.31^{a,b}$	$55.24 \pm 3.72^{a,b}$	67.24 ± 5.67^a	28.43 ± 4.04^c	$49.96 \pm 6.91^{a,b}$	39.68 ± 6.07^b

¹ Values are means \pm SEM over all six test days. a,b,c Means with no common letter differ significantly between subjects, $P \leq 0.05$, SNK-test.

² The supplement was consumed with a standard meal with or without added dietary fiber.



here (see below). In both studies the relative effect of dietary fiber on β -carotene bioavailability was of interest and there is no evidence of a much different fiber effect during the decreasing phase of β -carotene plasma response compared to increasing phase. Despite its limitations, the AUC method used should be suitable to determine relative effects of dietary constituents such as dietary fiber on the absorption of carotenoids and α -tocopherol within a repeated measurement study design where each subject serves as its own control.

Most studies on carotenoid bioavailability were performed using β -carotene. Besides β -carotene, α -carotene, lutein, zeaxanthin, lycopene and cryptoxanthin are most commonly consumed in a typical Western diet and are most prevalent in human plasma (Rock and Swendseid 1992, Yong et al. 1994). Absorption interactions between the different carotenoids and even between β -carotene and α -tocopherol were evident in several studies, although not all studies gave comparable results (Clark et al. 1998, Fotouhi et al. 1996, Johnson et al. 1997, Kostic et al. 1995, Nierenberg et al. 1994, O'Neill and Thurnham 1998, Paetau et al. 1997, Van den Berg and Van Vliet 1998, Xu et al. 1992). A mixture of different carotenoids and α -tocopherol was administered in the present investigation. However, the amount of the single substances (mg/d) may be about 10-fold higher than found in the diet of different populations while the proportions between the supplemented substances may be realistic, except for canthaxanthin (Riedl et al. 1997, Yong et al. 1994). Due to the commercial availability of a formulation suitable for administration in humans, canthaxanthin was added to the mixture while α -carotene and cryptoxanthin were missing. In other studies on carotenoid absorption after a single oral dose, comparable or even higher dosages were used (Brown et al. 1989, O'Neill and Thurnham 1998, Rock and Swendseid 1992, Van Vliet et al. 1995).

Five types of dietary fiber were given to the subjects at a dose of $0.15 \text{ g} \cdot \text{kg} \text{ bw}^{-1}$. The rationale for the selection of these fibers was to include members of the main types of fibers that are found in the diet, either naturally occurring or as ingredient used in the food industry. Pectin, guar (from the group of gums) and alginate (from the group of mucialges) were chosen as representatives of the group of water-soluble dietary fiber components. The intention to use hemicelluloses and lignins besides cellulose as the most important members of the group of water-insoluble types could not be realized because suitable preparations for administration to humans were not available. Instead, wheat bran, containing both hemicellulose and lignin, was used. A description of the properties and main dietary sources of the fibers can be found elsewhere (Kritchevsky and Bonfield 1995). The actual fiber doses administered ranged between 7.8 and 10.4 g per meal, representing about one-third of dietary intake recommendations per day (30 g/d) and can be judged as a reasonable amount of dietary fiber in a single meal. Consequently, the important issue for the evaluation of the effect of dietary fiber on the plasma response of supplemented antioxidants may be the interaction between the high actual antioxidant doses and the "normal" fiber dose.

Results from studies in chickens suggested that various types of dietary fiber (hemicellulose, lignin and pectin) reduce

FIGURE 2 Dietary fiber effect on the individual plasma response ($\text{AUC}_{24\text{h}}$) of β -carotene, lycopene, lutein, canthaxanthin and α -tocopherol to their supplementation in six healthy young women (subjects I–VI). The combined supplement was taken together with a standard meal with or without added dietary fiber. For significant differences between subjects, see Table 4. $\text{AUC}_{24\text{h}}$, areas under curves calculated after following for 24 h.

the bioavailability of β -carotene (Erdmann et al. 1986). In humans, Rock and Swendseid (1992) demonstrated that adding pectin (12 g) to a meal decreases plasma β -carotene response to a single dose of purified β -carotene (25 mg) by more than one-half. This finding was used to interpret the lower bioavailability of β -carotene from different food sources compared to purified β -carotene supplements (Brown et al. 1989, Rao and Rao 1970). The results of the present study fit well with those published by Rock and Swendseid (1992). When consumed with pectin, the mean AUC_{24h} of β -carotene decreased by 42% (Table 3). Similar to the findings for β -carotene, pectin supplementation decreased lycopene and lutein absorption by about 40% (Fig. 1). In the case of β -carotene, water-soluble types of dietary fiber had a stronger effect on relative bioavailability than did water-insoluble fibers. This tended to be true also for canthaxanthin. However, no distinction between the effects of water-soluble and water-insoluble types of fiber can be made for lycopene, lutein and α -tocopherol.

None of the various types of dietary fibers significantly reduced the α -tocopherol AUC_{24h}. However, the strongest effects were achieved by adding alginate (27% reduction) and wheat bran (24% reduction). One study in humans reported that addition of hemicellulose (3.9 g) to the diet significantly reduced the plasma response to a 500 mg α -tocopherol supplement (Doi et al. 1983). According to Mongeau et al. (1986), wheat bran significantly decreased plasma response of α -tocopherol in rats. Pectin also was used as dietary fiber supplement in rats (de Lumen et al. 1982, Schaus et al. 1985). A decrease of α -tocopherol absorption was measured when pectin was >6 g/100 g of nonpurified diet while 3 g/100 g had no effect. In the present study with a dose of 0.15 g pectin \cdot kg bw⁻¹, i.e., on average 8.85 g per test meal or about 3 g/100 g of the test meal, the effective level might not have been reached.

As stated by Schneeman (1990), digestion and absorption of nutrients in the presence of dietary fiber reflect the normal physiological situation. The presence of dietary fiber in the gut may have an important function in maintaining the gastrointestinal system by regulating the rate and site of nutrient absorption. Various sources of dietary fiber can slow down the process of digestion and absorption of macronutrients, including fat (Eastwood and Morris 1992, Edwards 1990, Schneeman 1990). It seems likely that the physical properties of fibers such as particle size, viscosity, water-binding capacity, gel formation, and bile acid-binding capacity are responsible for the observed effects and might also affect carotenoid and α -tocopherol absorption. First, the absorption of the fat-soluble carotenoids is suspected to have an absolute requirement for bile salt micelles (El-Gorab et al. 1975, Hollander and Ruble 1978). It was shown for guar gum that this type of fiber effectively binds bile acids and phospholipids (Vahouny and Cassidy 1985) and reduces phospholipid concentration in the intestine through an increase in chyme volume (Gallagher and Schneeman 1986), both of which disturb micelle formation. Furthermore, dietary fiber may exert effects on micelle formation by affecting the activity of pancreatic enzymes (lipase) also involved in micelle formation (Schneeman 1990). Second, soluble fibers increase viscosity and volume of the intestinal contents, which can slow diffusion processes such as the diffusion of micelles to the absorptive surface of enterocytes (Phillips 1986). And third, dietary fiber influences the morphology of the small intestine, including alterations in intestinal cell renewal (Sigleo et al. 1984). It was suggested that some carotenoid is held in the enterocytes and released in response to subsequent meals (Furr and Clark 1997). With an enhanced enterocyte turnover, more carotenoids may leave

the small intestine as constituents of desquamated mucosal cells. This effect may gain importance with chronic feeding of dietary fiber but not with their single supplementation.

Differences in molecular structure and polarity of the carotenoids and α -tocopherol might also help to explain the different effects of the various kinds of dietary fiber. However, since dietary fiber presents only few, if any, functional groups, the mechanism is probably an indirect one, again dealing with disturbances in micelle formation. The physical positioning of the various carotenoids within the mixed micelles depends on their polarity. Hydrocarbon carotenoids (β -carotene, lycopene), nonpolar compounds, may accumulate in the hydrophobic core of the micelles, while the more polar xanthophylls (lutein, canthaxanthin) may be located on the surface (Borel et al. 1996). However, nearly equal mean decreases of the AUC_{24h} of lycopene and lutein by the tested kinds of dietary fiber do not support these suggestions. On the other hand, the absorption of canthaxanthin, a carotenoid with a keto group, and α -tocopherol, revealed a different response to fiber supplementation compared to the other carotenoids. To some extent, the molecular characteristics of the target antioxidant may be important. Further, chemical and physical structures of the fibers specifically affect interactions with the individual carotenoids providing hydrocarbon, alcohol or ketone groups in the molecule. Possible binding mechanisms such as the formation of hydrogen bonds or hydrophobic interactions should be investigated.

In conclusion, it seems that the measured effects of various types of dietary fiber on the relative absorption of different carotenoids and α -tocopherol given in a mixture with a standard meal cannot be explained by a single criterion. Instead, they may be the result of many complex interactions. However, the strong decrease in the relative absorption of β -carotene (especially by water-soluble dietary fibers), lycopene and lutein (by all tested types of dietary fiber) points out the important effect of fiber in the human diet on carotenoid bioavailability.

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